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Review

Regulation of C/EBPβ and resulting functions in cells of the monocytic lineage

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ABSTRACT

Monocyte/macrophages play an important role in orchestrating the immune response. The present review refers to C/EBPβ, which is a key transcription factor regulating monocytic gene expression. Following a general introduction to C/EBPβ, this article focuses on activators and regulators of the C/EBPβ system in monocytic cells, including differentiating agents, cytokines, and bacterial products as well as associated signaling pathways. Furthermore, C/EBPβ target genes in monocytic cells are summarized and resulting functions are described, including regulation of proliferation and differentiation as well as orchestration of processes of mainly the innate immune response. In addition, a variety of disease stages are described in which a dysregulation of the C/EBPβ system may be involved. A detailed knowledge of the C/EBPβ system in monocytic cells may help to further understand the difference between inflammatory and malignant proliferation as well as additional regulatory facets of innate immunity.

Contents

1. Introduction

Monocytes are bone marrow-derived mononuclear cells giving rise to macrophages and dendritic cells (DC) with which they are forming

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the mononuclear phagocyte system [1,2]. Thus, they are part of the cellular arm of the innate immune system representing an important subgroup of the leukocytes [1]. Moreover, by covering antigen presentation, monocytic cells connect innate and adaptive immunity. Originating from hematopoietic stem cells, monocytes arise in the interplay of proliferation and differentiation. The monocytic development in the bone marrow comprises several premonocytic stages including multipotent progenitors (MPP; without self-renewal capacity), common myeloid progenitors (CMP; the source for all myeloid cells), and the granulocyte/macrophage progenitor (GMP) [3]. The further differentiated monocyte/macrophage and dendritic cell progenitor (MDP) has lost its granulocyte-forming potential but provides the basis for monocyte development [1]. Differentiated monocytes translocate to the blood where they are circulating in an inactive steady state, patrolling on the endothelial cells [1,4]. In humans, three monocytic subsets were identified: classical monocytes ($CD14^{++}CD16^{-}$), intermediate monocytes ($CD14^{++}CD16^{+}$), and non-classical monocytes ($CD14^{+}CD16^{++}$) [5,6]. Following emigration from the blood vessels into the respective tissue, monocytes may differentiate under physiological conditions into resident macrophages and DC (e.g. forming alveolar macrophages in the lung, Kupffer cells in the liver, white-pulp and metallophilic macrophages in the spleen, or microglia in the central nervous system) which are involved in tissue homeostasis [1,4,7]. When invading inflamed or damaged tissue, monocytes conduct initial proinflammatory and antimicrobial processes [8]. Subsequently, these monocytes may either efficiently differentiate within hours into inflammatory macrophages or DC [1,4] or into alternatively activated macrophages supporting tissue remodeling, wound repair, and immunomodulation [1]. Locally, monocyte/macrophages potentiate the immune response by cytokine and chemokine production, phagocytosis, and/or antigen presentation [4,9]. Finally, degenerated or transformed monocytic cells contribute to the development and/or progression of disease states such as leukemia [3], sepsis and inflammatory diseases [10] as well as persistence of microbial infections [6,8]. C/EBPβ represents one of the key transcription factors involved in the regulation of monocytic gene expression [11,12]. A variety of monocytic key features and functions such as proliferation/differentiation as well as orchestration of the immune response and phagocytosis, but also certain pathophysiological events such as acute myeloid leukemia (AML) or inflammatory processes, e.g. sepsis and bacterial/viral infections, appear to be (dys) regulated or modulated by C/EBPβ [11,12].

2. The transcription factor C/EBPβ

2.1. General aspects

CCAAT/enhancer-binding protein β (C/EBPβ) is a member of the C/EBP family of transcription factors currently consisting of 6 members: C/EBPα, C/EBPβ (also known as NF-IL6, IL6-DBP, CRP2, NF-M, AGP/EBP, ApC/EBP, or TCF5), C/EBPγ, C/EBPδ, C/EBPε, and C/EBPξ (for review see [11,13,14]). The founding member of the family, C/EBPα, was first described in 1986 as a protein purified from rat liver binding to double stranded viral DNA sequences exhibiting CCAAT-motifs [15,16] and further studies led to the further characterization of C/EBPα and the subsequently discovered family members [11,14,17]. C/EBPβ was first described in 1990 as a basic leucine zipper (bZIP)-structured factor binding to the interleukin 1 (IL-1)-responsive element in the IL-6 promoter (thus initially denoted as nuclear factor for IL-6 (NF-IL6)) and characterized by high C-terminal homology to C/EBPα [18].

In addition to other tissues (liver, lung, spleen, kidney, etc.), C/EBPβ is highly expressed in myelomonocytic cells and macrophages [19–22]. A variety of extracellular signals such as differentiation- or proliferation-inducing agents, hormones, cytokines and inflammatory substances as well as bacterial and other microbial products can act as activators or inhibitors of C/EBPβ *via* distinct signal transduction pathways [11], (see Section 3). The expression and/or activation of C/EBPβ is regulated in a complex way, e.g. by transcriptional mechanisms, mammalian target of rapamycin (mTOR)-mediated alternative translation, post-translational modifications, and protein–protein interactions, and have been extensively reviewed elsewhere [13,23,24]. Following activation, several classes of genes are induced or repressed by C/EBPβ: cytokines, chemokines, their respective receptors, and other proinflammatory genes, proproliferative or differentiation-related markers as well as metabolic enzymes [11]. Consequently, relevant cellular functions such as proliferation [21,22], differentiation [20,22,25], metabolic regulation [26,27], and orchestration of the immune response (e.g. by cytokine production or phagocytosis [28–30]) are affected by C/EBPβ. Moreover, due to its cellular relevance C/EBPβ may be involved in the pathogenesis of common diseases, e.g. cancer, hyper-/hypo-inflammation, and bacterial/viral infections [11,12].

2.2. Expression of C/EBPβ

The C/EBPβ promoter contains a TATA box and binding sites for several transcription factors regulating C/EBPβ mRNA expression including C/EBPβ itself [31–33], signal transducer and activator of transcription 3 (STAT3) [34], specificity protein 1 (Sp1) [35], members of the cAMP-responsive element binding protein (CREB)/activating transcription factor (ATF) family [35,36], early growth response 2 (EGR2) [37], Fos-related antigen 2 (Fra-2) [38], sterol-regulatory element-binding protein 1c (SREBP1c) [39], myoblastosis transcription factor (Myb) [40], and retinoic acid receptor α (RARA) [41]. The human gene coding for C/EBPβ (CEBPB, chromosomal localization: 20q13.13) is encoded by a single exon [42,43]. The yielding single mRNA (gene bank accession number NM_005194.2) spans a total of 1837 bases (overall G/C content: 66%), including 195 bases 5′UTR (G/C content: 77%), 1038 bases coding for C/EBPβ protein (incl. stop codon, G/C content: 74%), and 604 bases 3′UTR (G/C content: 50%).

The C/EBPβ mRNA half-life is approx. 40–60 min [44,45] but may be altered by mRNA stability-modifying proteins, especially HuR which binds the 3′UTR of the C/EBPβ mRNA and prolongs its half-life up to 110 min [45]. In addition, binding of HuR to the C/EBPβ mRNA may result in nuclear retention of the mRNA including the limitation of C/EBPβ translation in the cytosol [46]. Recently, it has been shown that C/EBPβ mRNA-bound HuR also plays a key role in the sequestration of C/EBPβ proteins from kinase-containing perinuclear cytosolic compartments thus preventing C/EBPβ proteins from activation [47].

Translation of the CEBPB mRNA results in the synthesis of three C/EBPβ isoforms: liver-enriched activating protein* (LAP* or C/EBPβ1), liver-enriched activating protein (LAP or C/EBPβ2), and liver-enriched inhibitory protein (LIP or C/EBPβ3) [13,48]. Human LAP* is a 44 kDa protein consisting of 345 amino acids (murine: 38 kDa/296 aa) and LAP is only slightly shorter (human: 42 kDa, 322 aa; murine: 34 kDa/275 aa). Both proteins exhibit a half-life of about 2 h. In contrast, LIP represents a markedly truncated C/EBPβ version (human: 20 kDa, 147 aa; murine: 20 kDa, 145 aa) with a half-life of approx. 8.5 h.

The formation of the different isoforms is mainly based on alternative translation affecting three different in-frame initiation codons at the positions +196 (for LAP*), +265 (for LAP), and +790 (for LIP) of the human mRNA [42,43]. This procedure depends on (i) weak Kozak consensus sequences around LAP* and LAP initiation codons in combination with an optimal Kozak context for LIP [42,43], (ii) an ex-frame upstream open reading frame (uORF; position +225) which is essential for LIP expression [42,43], and (iii) the modulation of the translation machinery by translation initiation factors (especially eIF-2α and eIF-4E) [42] or CUGBP1 [49] combined with leaky scanning of the ribosome [50,51]. In addition to alternative translation, the generation of LIP through directed proteolytic cleavage of the larger C/EBPβ isoforms has been discussed [52], presumably effected by μ-calpain [53] or the proteasome [54]. However, since protein isolation is sometimes accompanied by proteolytic

cleavage of full-length C/EBPβ *in vitro* [55], the *in vivo* relevance of this phenomenon has not been fully elucidated yet.

2.3. Structure and function of C/EBPβ

The C/EBPβ protein structure is characterized by an N-terminal section mainly harboring up to three transactivation domains (TAD1-3; absent in LIP) and two regulatory domains (RD1 and 2 in LAP* and LAP; in LIP the RD2 region is complete while RD1 exists in a truncated form) [11,56]. C/EBPβ and the other C/EBP proteins share a highly conserved C-terminus (>90% sequence identity) which contains a basic DNA binding domain adjacent to a leucine zipper-type dimerization domain (for review see [11,13,14,17,24]). Following dimerization, the DNA binding domains mediate binding activity to their corresponding DNA binding sequences (ideal binding sequence: ATTGCGCAAT, a variant of the general C/EBP consensus sequence $^{A}/_{G}$TTGCG$^{C}/_{T}$AA$^{C}/_{T}$) [57] as well as protein homo- and/or heterodimerization [24].

Various post-translational modifications and events including phosphorylation, acetylation, methylation, sumoylation, and proteolysis strictly regulate dimerization, cellular localization, DNA binding, and the transactivation activity of C/EBPβ [11,13,24,58]. Furthermore, C/EBPβ transactivation capacity is based on the presence (C/EBPβ-LAP* and -LAP) or absence (C/EBPβ-LIP) of intrinsic transactivation potential, the structure of the respective transactivating isoform (C/EBPβ-LAP or C/EBPβ-LAP*), the relative amount of actually present isoforms (LAP/LIP ratio), as well as on the appropriate dimerization/interaction partners [11,13,24]. C/EBPβ-dependent target gene activation further depends on the composition of target gene promoters as well as on the respective cell-, tissue-, and species-specific conditions [11,13,24]. In consequence, LAP*, LAP, and LIP exhibit distinct functions.

In general, LAP* is a less potent activator of gene transcription than LAP with respect to pure transactivation activity [56], presumably based on the formation of an additional disulfide bond [59]. However, its extended N-terminus appears to mediate the recruitment of the 'switch/sucrose nonfermentable' (SWI/SNF) nucleosome remodeling complex and this interaction may not only enable the activation of usually silenced genes but also facilitate the interaction of LAP* with the myeloid-specific transcription factor c-Myb [60]. Therefore, in the respective context, the cooperation with other transcription factors and regulators renders LAP* a more effective (co)regulator of myeloid genes than LAP [60]. In contrast, LIP is generally regarded as a dominant inhibitor of transcriptionally active C/EBPs including LAP* and LAP [61].

In addition to dimerization with proteins of the same family, C/EBPβ is also able to interact/cooperate with other proteins thus expanding the accessible DNA binding motifs and modifying C/EBP protein functions and specificities like initiation of target gene transcription [24]. Known protein interaction partners of C/EBPβ are other bZIP-structured transcription factors, e.g. members of the Jun/Fos [62] and the CREB/ATF families [63]. In addition, C/EBPβ may interact with non-bZIP proteins such as nuclear factor-κB (NF-κB) subunits p65 [29] and p50 [64], glucocorticoid receptor [65], retinoblastoma protein (Rb), E2F [66,67], c-Myb [60], SWI/SNF, protein arginine N-methyltransferase-4 (PRMT4)/coactivator-associated arginine methyltransferase 1 (CARM1) [68], p300/CREB-binding protein (CBP) [69], the mediator complex [51], PU.1 [25,70], death-associated protein 6 (Daxx) [71], C-repeat binding factors (CBF), and runt-related transcription factor (Runx) proteins [72]. Moreover, by adopting the function of a binding partner for other transcription factors (especially NF-κB, glucocorticoid receptor, and Runx2), LIP may also contribute to gene expression *via* accessing certain promoters [13].

3. Activators and modulators of C/EBPβ in monocytic cells and associated signaling pathways

In myelomonocytic cells and monocyte/macrophages, C/EBPβ is regulated by a variety of differentiation- and proliferation-inducing agents, cytokines and inflammatory substances as well as bacterial and other microbial products (Fig. 1).

3.1. Differentiation-inducing agents

In human promyelocytic leukemia HL60 cells stimulated with 12-O-tetradecanoylphorbol-13-acetate (TPA; also known as phorbol 12-myristate 13-acetate, PMA), the expression of C/EBPβ mRNA is strongly induced [73]. Avian leukemia virus E26-infected multipotential stem cells from chicken which were differentiated towards the myelomonocytic lineage using PMA exhibited increased levels of C/EBPβ protein [20]. In addition, the combination of PMA and lipopolysaccharide (LPS) enhanced C/EBPβ promoter activity and transcriptional initiation in a CREB/ATF- and Sp1-dependent manner resulting in increased expression levels of C/EBPβ mRNA [35] and the larger C/EBPβ proteins in histiocytic leukemia U937 cells [73]. Moreover, the stimulation of chicken macrophages with TPA caused the translocation of C/EBPβ to the nucleus [20]. In human premonocytic THP-1 cells, a PMA-induced increase in all C/EBPβ isoforms occurs together with an increase in the LAP/LIP ratio [22,25]. Moreover, stimulation of chicken macrophage HD11 cells with PMA as well as upregulation of cAMP by incubation with the adenylyl cyclase activating component forskolin or phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX), respectively, increased stability and level of C/EBPβ mRNA [44]. Consequently, PKA- as well as PKC-dependent intracellular signaling pathways appear to positively regulate C/EBPβ expression [74]. Interestingly, *in vitro* differentiated (*via* cell culture for up to 11 days) primary human monocytes exhibit constant C/EBPβ mRNA levels [25].

Using all-trans-retinoic acid (ATRA) or a combination of vitamin D_3 (Vit-D_3) and transforming growth factor β (TGF-β), U937 cells show both enhanced expression of C/EBPβ protein and enhanced binding activity at C/EBPβ target gene promoters including increased activation of a C/EBPβ target gene promoter-dependent reporter construct [75,76]. Interestingly, following ATRA stimulation, the increase in C/EBPβ was shown to be influenced by the availability of the N-Myc downstream-regulated gene 1 (NDRG1) protein [77]. In ATRA-treated human acute promyelocytic leukemia NB4 cells, C/EBPβ mRNA and (LAP*-/LAP-) protein expression as well as C/EBPβ DNA binding activity were induced while C/EBPβ mRNA half-life was unaffected [41]. In this case, activation of the C/EBPβ promoter appears to depend on a positive regulation by promyelocytic leukemia-retinoic acid receptor α (PML-RARA) [41]. Moreover, the influence of ATRA on C/EBPβ expression is significantly enhanced in NB4 cells when combined with dehydroepiandrosterone (DHEA) [78]. Synthetic retinoids which specifically bind to retinoic acid receptors α and β, like tamibarotene (tami) and the tamibarotene dimethylaminoethyl ester A112, are also able to induce C/EBPβ protein expression in a dose-dependent manner as shown in NB4 cells [79]. In HL60 cells, it could be shown that the deltanoid 1,25-dihydroxyvitamin D_3 ($1,25(OH_2)D_3$) enhances C/EBPβ mRNA and protein levels *via* the activation of Raf, mitogen-activated ERK kinase (MEK)/extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK), respectively [80–82]. These processes are accompanied by an upregulation of the vitamin D receptor (VDR) as well as the Ras/mitogen-activated protein kinase (MAPK) pathway activating scaffold protein kinase suppressor of Ras (KSR-1) which presumably amplifies Raf-dependent signal intensity even in the presence of low deltanoid concentrations [81]. In this context, p38 MAPK appears to repress C/EBPβ mRNA and protein expression, since both are induced following p38 inhibition [80]. Recent findings demonstrate that the treatment of HL60 cells with the pentacyclic triterpenoid compound ursolic acid (UA) also induces C/EBPβ mRNA and protein expression by activating the ERK pathway [83].

3.2. Proliferation-inducing agents

Analyses in v-Myb and epidermal growth factor receptor (EGFR/HER1)-transduced chicken myeloblasts showed that the stimulation

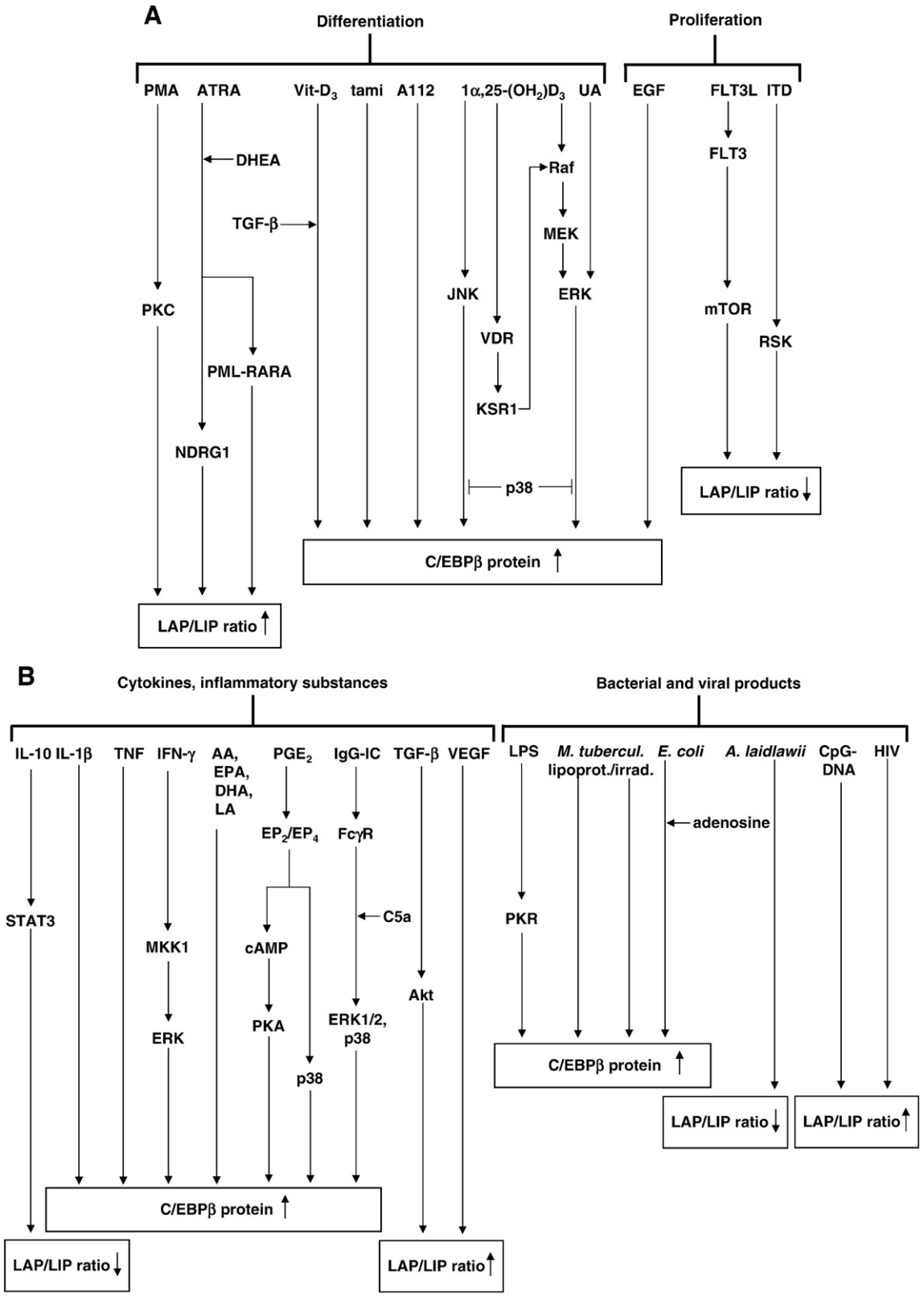

Fig. 1. Activators and modulators of the C/EBPβ system. (A, B) Depicted are conditions or molecules inducing a change in the expression of C/EBPβ-LAP*/LAP or LIP proteins (either in whole cell or cytosolic/nuclear extracts). In addition, associated signaling pathways are shown. (C) Conditions/molecules and signaling pathways are shown which activate or inhibit the C/EBPβ system (mRNA, phosphorylation, DNA binding activity, and transactivation activity). Modulators which are mentioned in A or B are not enlisted. Further details are described in Section 3. For abbreviations see text.

with epidermal growth factor (EGF) – which is known to mediate monocyte chemotaxis and macrophage proliferation [84] – enhanced nuclear translocation of C/EBPβ protein [20]. An increased nuclear localization was also observed in chicken macrophages following transduction with the leukemia virus MH2 which contains the v-mil (i.e. Raf) oncogene [20]. Murine 32D haematopoietic cells stably

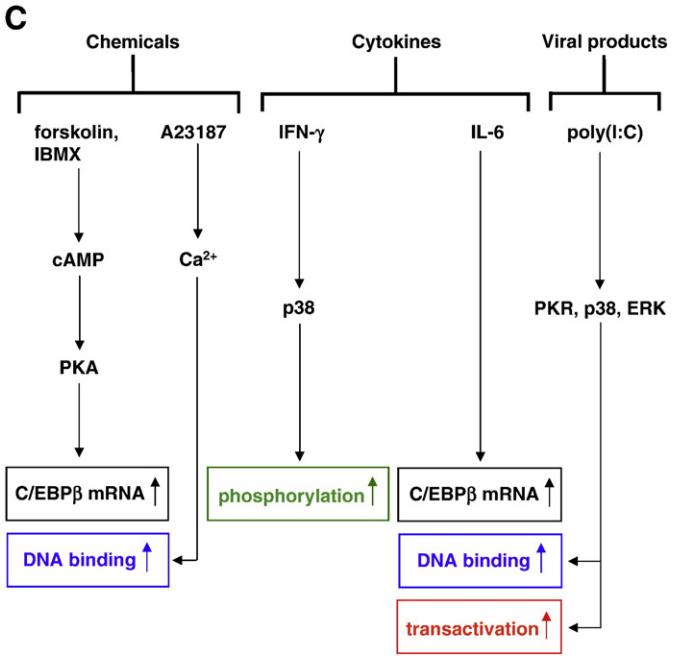

Fig. 1 (*continued*).

transfected with the wildtype (WT) fms-like tyrosine kinase receptor-3 (FLT3) and stimulated with its ligand FLT3L are characterized by elevated C/EBPβ-LIP protein expression accompanied by a decreased LAP/LIP ratio [21]. Both effects are also visible in cells possessing a constitutively activating FLT3 mutation, i.e. FLT3 including an internal tandem duplication (ITD) (human AML cell lines MV4-11, MOLM-13, and PL-21 as well as murine FLT3-ITD-positive 32D cells). Despite elevated protein levels in ITD-positive cells, unstimulated FLT3-WT- and FLT3-ITD-positive cells exhibit comparable C/EBPβ mRNA levels [21]. Interestingly, FLT3-ITD-positive and FL-stimulated FLT3-WT cells initiate different signaling cascades (mTOR-dependent vs. p90 ribosomal S6 kinase (RSK)-dependent) [21].

3.3. Cytokines and inflammatory substances

The stimulation with IL-6 strongly induces C/EBPβ mRNA expression in murine M1 myeloid leukemia cells [62,73] and an IL-6-driven increased binding to and activation of the myeloid differentiation factor 2 (MD-2) promoter by C/EBPβ was observed in human THP-1 cells [70]. Analyses performed in murine J774.2 macrophage-like cells revealed that tumor necrosis factor (TNF), IL-1, and interferon γ (IFN-γ) induced C/EBPβ mRNA and protein expression as well as C/EBPβ DNA binding activity [85]. In the murine macrophage-like cell line RAW 264.7, it could be shown that the IFN-γ-dependent effects on expression and activation of C/EBPβ protein are mediated *via* activation of mitogen-activated protein kinase kinase 1 (MKK1) and ERK [86]. In human U937 cells, TGF-β stimulation as well as co-cultivation with TGF-β producing mesenchymal stem cells results in a moderate induction of both C/EBPβ mRNA and LAP (but not LIP) [87]. This response appears to be mediated by TGF-β-dependent protein kinase B (PKB)/Akt activation. Following phorbol ester-induced differentiation of HL60 cells to macrophages, these cells strongly activate the C/EBPβ promoter in response to anti-inflammatory IL-10, presumably *via* STAT3 recruitment, resulting in a massive LIP induction while LAP is only slightly increased [88]. Conversely, treatment of THP-1 cells with vascular endothelial growth factor (VEGF) differentially affects the C/EBPβ isoforms by negatively regulating the concentration of C/EBPβ-LIP thus increasing the LAP/LIP ratio [89]. In primary human monocytes as well as in the human AML cell line Mono-Mac 1, activation of the prostaglandin receptors E-prostanoid 2 (EP_2) and 4 (EP_4) following stimulation with prostaglandin E_2 (PGE_2) yields elevated levels of cAMP and the subsequent activation of PKA and the p38 MAPK pathway resulting in nuclear translocation and enhanced binding of C/EBPβ to its recognition sequence [90]. Recently, it has been shown that C/EBPβ mRNA and protein are induced, C/EBPβ DNA binding activity is enhanced, and C/EBPβ-dependent gene expression is increased in RAW264.7 cells treated with IgG immune complexes (IgG-IC) [91]. Mechanistically, this effect is presumably mediated *via* Fcγ receptors, includes the kinases ERK1/2 and p38 MAPK, and is further enhanced in the presence of the complement component 5a (C5a). Increased C/EBPβ mRNA and protein levels can also be induced in U937 cells by addition of polyunsaturated fatty acids such as α-linolenic acid (LA), arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) [92].

3.4. Bacterial and other microbial products

An incubation of human peripheral monocytes as well as rat peritoneal macrophages with LPS markedly increased the C/EBPβ mRNA expression [73,93] and in primary chicken macrophages, an induction of C/EBPβ protein translocation to the nucleus could be demonstrated [20]. Further analyses in murine J774.2 and chicken HD11 cells showed an enhanced C/EBPβ gene transcription in response to LPS accompanied by elevated protein synthesis and DNA binding activity [44,85] while C/EBPβ mRNA half-life remained unaffected [44]. Equivalent experiments performed with U937 cells suggest a regulatory involvement of protein kinase R (PKR) in enhanced C/EBPβ protein expression, because PKR inhibition markedly reduced LAP*/LAP levels [94]. However, since the LPS-driven enhancement of C/EBPβ DNA binding activity is mimicked by application of forskolin and the calcium ionophore A23187 (especially when combined), cAMP-dependent PKA and Ca^{2+}-dependent signaling pathways also appear to be of importance for C/EBPβ regulation [95]. In primary human blood monocytes, the stimulation with IFN-γ elevated p38-mediated C/EBPβ phosphorylation and treatment applying a combination of LPS and IFN-γ increased the DNA binding activity of C/EBPβ [96]. In addition, the activation of a (C/EBPβ-regulated) 1α-hydroxylase promoter-dependent reporter construct was increased in RAW264.7 cells following stimulation with LPS/IFN-γ. Remarkably, under these conditions C/EBPβ protein expression was not affected. In RAW 264.7 cells, the same treatment enhanced DNA binding activity of full length C/EBPβ, an effect which could be inhibited by application of α-melanocyte-stimulating hormone (α-MSH) [97].

Murine bone marrow-derived macrophages challenged with IFN-γ and/or the 19 kDa lipoprotein from *M. tuberculosis* exhibited increased C/EBPβ mRNA and LAP*, LAP, and LIP levels as well as intensified binding to C/EBPβ-dependent class II transactivator (CIITA) promoters I and IV [98]. Comparable results were obtained in RAW264.7 cells treated with irradiated *M. tuberculosis* and IFN-γ [99] or exposed to *Escherichia coli* [100]. The *E. coli*-dependent effects were further enhanced by treatment with adenosine [100]. Remarkably, *Acholeplasma laidlawii*-treated THP-1 cells exhibit a differential regulation of C/EBPβ isoforms since the expression of LIP is strongly induced while LAP remains constant [101]. In human bone marrow-derived macrophages, stimulation with immunostimulatory CpG-DNA resulted in increased C/EBPβ mRNA and protein expression (LAP*, LAP, and to a lesser extend LIP) in combination with enhanced DNA binding activity [102]. Treatment of human and murine primary monocytes and macrophages as well as RAW 264.7 cells with a dsRNA mimetic (polyinosinic:polycytidylic acid) leads to PKR, p38, and ERK activation and results in enhanced binding of C/EBPβ and activation of C/EBPβ target gene promoter-dependent reporter constructs, respectively [103]. Moreover; in granulocyte macrophage colony-

stimulating factor (GM-CSF)-treated and C/EBPβ-expressing macrophages, LIP expression is markedly increased following HIV infection [104].

4. Target genes of C/EBPβ in monocytic cells and resulting functions

Following activation of C/EBPβ and depending on the prevalent cellular context, the expression of the respective target genes is enhanced or repressed. Thus, many general and monocyte/macrophage-specific cell functions are regulated including proliferation and differentiation (Fig. 2). In addition, C/EBPβ plays a major role in the orchestration of the immune response, mainly the cellular branch of innate immunity. We will also focus on the involvement of C/EBPβ in leukemia, inflammation, and bacterial or viral infections.

4.1. Proliferation and differentiation

4.1.1. Proliferation

Proliferation and cell cycle progression appear to be among the cellular key features influenced by C/EBPβ in (myelo)monocytic cells [105]. FLT3L induces the small C/EBPβ isoform LIP in hematopoietic cells stably transfected with the FLT3-WT receptor thus decreasing the LAP/LIP ratio which is strongly associated with proliferation [21]. Corresponding data from LIP induction-deficient murine embryonic fibroblasts show a considerable reduction of cell duplication and proliferation marker expression (proliferating cell nuclear antigen and cyclins A1, A2, B1, E1, and E2) [106]. Interestingly, the complete absence of C/EBPβ differentially affects proliferation of cells from distinct developmental stages, influencing either early developmental stages (i.e. before spreading in a granulocytic and a monocytic lineage) or further differentiated cells: in C/EBPβ-deficient bone marrow-derived progenitor cells, the formation of myeloid colonies in methylcellulose as well as the number of generated cells were significantly reduced [107]. In these experiments, a variety of stimulating agents such as granulocyte colony-stimulating factor (G-CSF), GM-CSF, and IL-3 were applied. In contrast, in further differentiated cells, a C/EBPβ knock out generates a proproliferative situation [22,108]. C/EBPβ^{ko} mice are characterized by hyperplastic hematopoiesis and hypermyeloproliferation [108] and in C/EBPβ^{ko} macrophages, proliferation rates as well as the amount of cells exhibiting S or G_2/M phase markers are markedly enhanced [22]. This is presumably mediated by the larger C/EBPβ isoforms *via* different mechanisms: despite normally acting as transactivators, LAP* and LAP proteins repress the expression of transcription factor c-Myc in monocytic cells [22,83]. Thus, C/EBPβ decreases proproliferative c-Myc target genes such as cyclin D but increases cell cycle inhibitors like p27 which are inhibited by c-Myc [22]. Another effect was originally described in fibroblasts in which C/EBPβ associates with the Rb-E2F protein complex and contributes to Rb-dependent cell cycle arrest as well as downregulation of E2F target genes [66,67]. Comparable results were obtained in ATRA-differentiated [80] as well as C/EBPβ-transduced (pre)monocytic cells in which C/EBPβ interacts with Rb and/or E2F proteins ([22], K. Brand unpublished observations). Accordingly, predominant expression of LAP*/LAP tends to arrest the respective cells in the G_0/G_1 phase of the cell cycle and results in a reduced proliferative capacity of monocytic cells [22].

4.1.2. Differentiation

4.1.2.1. Induction of differentiation-associated genes and resulting functions. C/EBPβ-LAP* and -LAP have been shown to be considerably connected to monocytic differentiation [105]. The differentiation of (pre)monocytic cells is characterized by significant upregulation of C/EBPβ, especially LAP* and LAP [20,22,25,73,75,80,83]. This is also reflected by an increasing LAP/LIP ratio [22]. Both effects are also observed during differentiation of other cell types like hepatocytes [61,109] and adipocytes [110,111].

Besides the inhibitory effect of the larger C/EBPβ isoforms on (pre) monocytic proliferation (as described in Section 4.1.1), C/EBPβ is involved in the regulation of the expression of a variety of differentiation-associated genes including CD14 [75,80,83,87], macrophage-2 antigen (Mac-2), Fcγ receptor II (FcγRII) [112], monocyte-specific esterase [80], 1α-hydroxylase [96], and the cytoplasmic proline-rich tyrosine kinase 2 (Pyk2) [113]. The expression of other genes such as MD-2 and chitotriosidase (CHIT1) is also supported by C/EBPβ by acting as a cofactor of transcription factor PU.1 thus mobilizing its entire transcriptional capacity [25,70]. Similarly, in the transcription of chicken MER1-repeat-containing imprinted transcript 1 (mim-1) – which was early identified as positively influenced by C/EBPβ [40,114] – LAP* and LAP enhance c-Myb-dependent transcription by inducing chromatin opening presumably *via* recruitment of histone acetylating cofactors of the p300/CBP family (by LAP* and LAP) and/or the SWI/SNF chromatin remodeling complex (exclusively by LAP*) [68,115]. The presence of C/EBPβ and the activation of its maturation-associated target genes is attended by reduced proliferation, morphological changes [22,83], and an increasing antimicrobial capacity [83]. Although C/EBPβ^{ko} mice are able to generate macrophage-like cells, these cells exhibit a reduced functional potential since their activation in response to activating stimuli (e.g. LPS, bacteria) is impaired [108,116]. In immortalized macrophage-like cell lines derived from C/EBPβ^{ko} mice, the induction of differentiation/activation markers [112] as well as the development of a typical macrophage morphology is impaired [22]. Altogether, C/EBPβ-LAP* and -LAP appear to be key factors especially in terminal monocyte-to-macrophage differentiation.

4.1.2.2. Granulopoiesis and osteoclastogenesis. In progenitor cells exhibiting early stages, i.e. in cells providing the formation of both granulocytes and monocytes, LAP* and LAP are able to promote the differentiation of the granulocytic lineage [107,117]. Overexpression of C/EBPβ in murine primary bone marrow cells results in a reduction of myeloid progenitor cells in combination with enhanced formation of granulocytes, while LIP overexpression did not affect myeloid progenitor formation [117]. Moreover, in C/EBPβ^{ko} mice infected with *C. albicans*, a reduced amount of emergency granulopoiesis was shown [107]. In addition, in patients with congenital neutropenia, G-CSF significantly increased the expression of C/EBPβ in bone marrow-derived myeloid cells resulting in emergency granulopoiesis [118].

In contrast, the formation of osteoclasts from myeloid progenitor cells is enhanced by LIP but inhibited by the larger isoforms which is presumably based on the regulation of the osteoclastogenesis inhibitor MafB [119,120]. Comparable results were obtained in uORF-deficient mice which are not able to induce LIP expression, but show increased levels of MafB in combination with impaired osteoclastogenesis [106].

4.1.3. Leukemia

The role of C/EBPα for the development of leukemia has been extensively investigated [121,122]. In this chapter, however, we will focus on C/EBPβ which is also often directly connected to the development of different myelomonocytic leukemias [50,123,124]. For instance, C/EBPβ is dysregulated in a subtype of AML which is characterized by the constitutively activating ITD mutation of the FLT3 receptor [21]. In ITD-positive AML patients, enhanced LIP concentrations were observed in comparison to ITD-negative patients. Accordingly, ITD-positive monocytic cell lines (MV4-11, MOLM-13, PL21) exhibit significant LIP levels. LIP is also highly expressed in a variety of other leukemia cell lines, e.g. murine K562 erythroleukemia cells [125] or the anaplastic large cell lymphoma cell lines SUDHL-1, Ki-JK, and Karpas 299 [126]. Comparable results were also obtained in additional types of cancer such as breast cancer [127–130], as well as several carcinoma cell lines (e.g. human breast cancer cell line MCF7, human cervical carcinoma cell line HeLa) [126]. The larger C/EBPβ isoforms, however, are generally reduced in strongly proliferating cells, e.g. in bone marrow-derived cells during blast crisis of chronic myeloid leukemia (CML) patients [131].

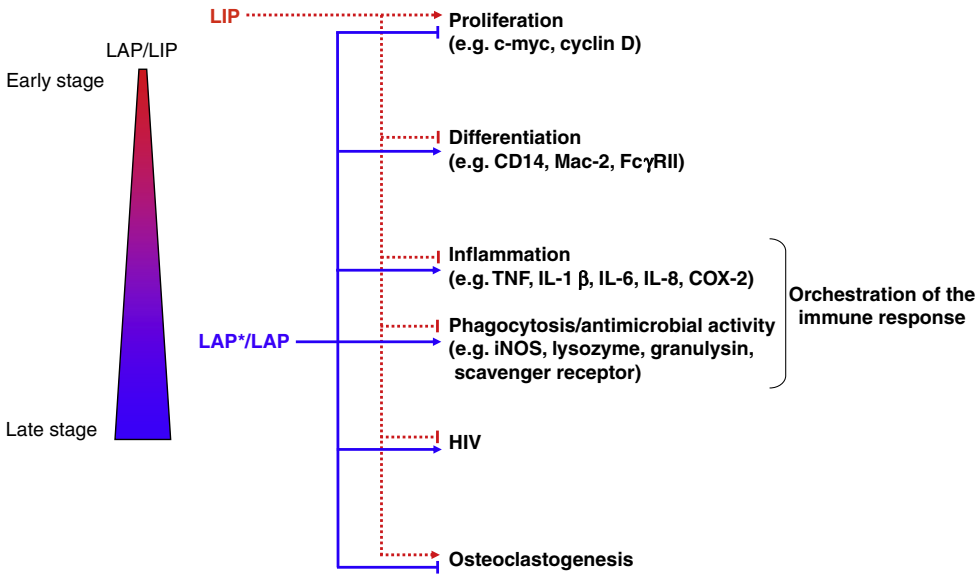

Fig. 2. C/EBPβ-associated functions and target genes in monocytic cells. The monocytic features and functions influenced either positively or negatively by LAP*/LAP (blue line) or LIP (red dotted line), respectively, including selected target genes of C/EBPβ are shown. In addition, the shift in the LAP/LIP ratio during monocyte development from early to late stages is illustrated. For abbreviations see text.

Moreover, in myeloblasts isolated from patients with t(1;11)(q21;q23) mixed lineage leukemia (MLL) which additionally harbor a chromosomal insertion interrupting CUGBP1, a protein influencing C/EBPβ translation, all C/EBPβ isoforms are virtually absent [132]. In contrast, the upregulation of LAP*/LAP reduces proliferation and induces differentiation of leukemia cells [41], thus suppressing leukemogenesis in the corresponding mouse model [133]. The induction of LAP*/LAP by ATRA in NB4 acute promyelocytic leukemia cells results in the differentiation of these less developed myelomonocytic cells towards a granulocytic cell type [41]. Accordingly, this approach fails in ATRA-resistant cells in which larger C/EBPβ isoforms could not be induced [41]. The treatment of primary AML cells with deltanoids [81], U937 leukemia cells with Vit-D_3 [134] or ATRA [77] or HL60 leukemia cells with 1,25$(OH_2)D_3$ [80] also induced an increase in C/EBPβ protein as well as the differentiation of these cells into monocytes. Consequently, treatment with Vit-D_3 derivates and/or analogs may represent a promising alternative therapeutical approach for leukemia patients who do not respond to a classical chemotherapy *via* the induction of C/EBPβ-supported differentiation [135].

4.2. Orchestration of the immune response

4.2.1. Regulation of inflammatory genes

Following the implementation of monocytic differentiation, C/EBPβ is associated with a variety of immunogenic features [28]. One important aspect of monocyte-associated immune response is the production of C/EBPβ-dependent cytokines and their receptors [11,28]. As shown in C/EBPβ^{ko} mice as well as different (pre)monocytic cell lines, C/EBPβ is involved in the regulation of the expression of TNF, IL-1β, IL-6, IL-12 p35, and IL-10 [100,112,136–139] as well as the receptors for macrophage colony-stimulating factor (M-CSFR) and IL-13 (IL-13RA1) [92,139]. This is consistent with early promoter studies in Jurkat cells for IL-6 and in murine P19 cells for IL-6 and IL-8 [18,140]. However, experiments performed in an alternative knock out model did not yield impaired TNF, IL-1, IL-6, IL-10, IL-12, macrophage colony-stimulating factor (M-CSF), and GM-CSF production [116]. The mRNA expression of G-CSF in peritoneal macrophages, however, was impaired in this model [116]. In the case of G-CSF, C/EBPβ-LAP appears to be the isoform with the strongest influence on gene expression, since murine LAP-deficient macrophages show virtually no LPS-induced G-CSF production [141]. The differences among selected knock out models may result from the analysis of different and functionally heterogeneous macrophage populations thus generating distinct phenotypes which may not be representative of all possible populations [112]. It has also been proposed that depending on the specific nuclear composition in C/EBP factors, C/EBPβ gene products might act as repressors either as the result of a prevalent function of the inhibitory counterpart LIP and/or because full length C/EBPβ is a weaker transcriptional activator than other C/EBP proteins [108]. However, in retransduced cells, C/EBPβ-dependent cytokine expression is (at least in part) recovered suggesting that impaired gene expression in the respective knock out model indeed appears to be effected by the absence of C/EBPβ [112]. IL-12 p40 appears to be negatively regulated by C/EBPβ since the absence of C/EBPβ results in the induction of this gene [112]. This inhibition was shown to be predominately mediated by C/EBPβ-LAP, since this protein is strongly upregulated in LAP-deficient macrophages in response to LPS/IFN-γ [141]. However, the more prevalent type of gene suppression is mediated by LIP, the dominant-negative variant of C/EBPβ [11], as shown for TNF [138], IL-6, and IL-10 [89].

In addition to cytokines, a variety of chemoattractants are positively or negatively regulated by C/EBPβ. C/EBPβ induces CC-motif chemokine receptor 7 [90] and upregulates the CXC chemokines IL-8 (as shown in fibrosarcoma cells [142]) and stromal cell-derived factor 1 (SDF1) [143] while CC-motif chemokine ligand 5 (CCL5/RANTES) and macrophage inflammatory protein 1β (MIP1β) appear to be downregulated [112]. In contrast to MIP1β, MIP1α and/or MIP2 have been shown to be not essentially [112,116] or even positively regulated by C/EBPβ [91] in different C/EBPβ^{ko} models [108,116,144].

Among further C/EBPβ-dependent proinflammatory genes are cylooxygenase 2 (COX2) as the generator of prostaglandin H_2 [145], the proinflammatory Ca^{2+}-binding proteins S100A8 [103] and S100A9 [103,146], CIITA, a transcriptional co-activator of MHC-II genes [98], and arginase I which contributes to the development of different inflammatory diseases [93,147]. Moreover, the NF-κB system is also influenced by C/EBPβ which enhances NF-κB-mediated signaling by reducing the level of the NF-κB inhibitor IκBα [30]. In addition, C/EBPβ contributes to the development of TNF tolerance: following repeated stimulation of monocytic cells with low doses of TNF, the stimulatory capacity of further and even higher doses of TNF for C/EBPβ target gene induction (e.g. IL-8) is inhibited [148]. In this context, C/EBPβ inhibits function and activity of NF-κB by impeding NF-κB p65 phosphorylation *via* direct protein-protein interaction [29]. This phenomenon is considered as

protective in sepsis but may also represent a paradigm for immunoparalysis. In C/EBPβ^{ko} macrophages, a reduced level of the scavenger receptor was determined [112]. In this context, it should be mentioned that it has been suggested that C/EBP proteins are also involved of the pathogenesis of the chronic inflammatory process of atherosclerosis. For example, it has been shown that oxidized low density lipoprotein induces the scavenger receptor in smooth muscle cells which correlates with activation of C/EBPβ [149]. In addition, it has been shown that C/EBP protein decoy oligonucleotides inhibit macrophage-rich vascular lesion formation in hypercholesterimic rabbits [150].

4.2.2. Bacterial infections

A significant reduction in the synthesis of phagocytosis-related proteins emerged in C/EBPβ-deficient monocyte/macrophages characterized by a decreased expression of receptors recognizing pathogen-associated molecular patterns (PAMPs) and/or supporting phagocytosis, e.g. the LPS co-receptor CD14 [75,87], FcγRII, Mac-2, and the above mentioned scavenger receptor [112]. Furthermore, antimicrobial C/EBPβ target genes such as inducible nitric-oxide synthase (iNOS) [93,112,145,151], lysozyme [44,95], hepcidin [99], and granulysin [101] are reduced when C/EBPβ is absent or inhibited. Consequently, C/EBPβ^{ko} mice are characterized by enhanced susceptibility to *Candida albicans*, *Listeria monocytogenes*, and *Salmonella typhimurium* infections [108,116]. In this model, an appreciable amount of pathogens escapes from the phagosome to the cytoplasm [116] reflecting a diminished (but not completely omitted) antimicrobial activity. Tumor cytotoxicity and tumoristatic activity against mastrocytoma cells were also markedly impeded [116]. The amount of phagocytosed particles, however, was not affected [112,116] and intracellular bacterial killing does not appear to depend necessarily on the presence of LAP, since LAP-deficient macrophages show no impairment of this cellular feature [141]. In murine bone marrow-derived macrophages, knock out of the A20-binding inhibitor of NF-κB (ABIN1), an antiinflammatory factor within TLR-dependent signaling which restricts C/EBPβ expression and activity, results in an excessive upregulation of C/EBPβ and its proinflammatory target genes (e.g. G-CSF and S100A8) in response to CpG-DNA [102]. In the respective mice, this provokes severe inflammation, considerable expansion of myeloid cells, and infiltration of leukocytes in liver, lung, and kidney [102].

4.2.3. Viral infections

C/EBPβ also plays an important role in viral infections (for a detailed review see [12]). The influence of C/EBPβ on viral infections has been especially studied in human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) infections. In general, C/EBPβ-LAP*/LAP appear to enhance long-terminal repeat (LTR)-dependent transcription and viral replication of several viruses, e.g. HIV-1 [12], SIV [152], and Karposi's sarcoma-associated herpesvirus (KSHV) [153]. In particular, LAP* and LAP support the recruitment of cofactors to the HIV-1 LTR and interact with histone acetyltransferase complexes thus presumably facilitating HIV-1 chromatin remodeling as shown in U937 cells [154,155]. By positively regulating viral replication, LAP*/LAP support secondary infections but appear to be dispensable for the initial infection of monocyte/macrophages since their inhibition by LIP overexpression has no protective effect on primary infections [155]. In this context, not only C/EBPβ-supported HIV-infection of premonocytic cell lines (U1 and U937) but also primary macrophages may be enhanced by adjacent IL-6 and intercellular adhesion molecule 1 (ICAM-1) expressing endothelial cells [156]. In M-CSF-induced macrophages, LAP* (and to a lesser extent LAP) expression is detectable and following HIV-1 infection, a large amount of viral particles is produced while C/EBPβ expression was not affected [104]. In contrast, not only in GM-CSF-induced macrophages LAP*/LAP but also LIP are strongly expressed and the LIP concentration markedly increases following HIV infection resulting in the repression of viral protein expression [104]. The inhibitory effect of LIP on HIV replication was also observed in PMA-differentiated macrophages (derived from THP-1 and HL60 cells) in which IFN-γ- or IL-10-induced LIP expression lead to inhibition of LTR-dependent viral transcription and replication [88,157]. Comparable results have been obtained in the SIV model [158]. Hence, the protective effects of LIP may be utilized in anti-HIV therapy: for instance, it has been shown that murabutide, a muramyl dipeptide derivate, suppresses HIV replication by inducing LIP expression in macrophages derived from PMA-stimulated THP-1 cells [159]. Another mechanism is mediated by the protein p27^{sj} isolated from *Hypericum perforatum* which associates with C/EBPβ proteins as well as the viral transactivator protein Tat [160]. Since the formed protein complex is unable to enter the nucleus but accumulates in a perinuclear cytoplasmic compartment, HIV transcription and replication are repressed [160,161].

5. Concluding remarks

In conclusion, the C/EBPβ system is involved in regulating the development of (myelo)monocytic cells as well as specific functions of this cell type. The short C/EBPβ isoform LIP appears to be associated with proliferation at myelomonocytic progenitor stages, while LAP* and LAP may favor the formation of the granulocytic lineage at this stage. At later developmental stages, i.e. differentiation from premonocytic cells to monocytes and further to macrophages, the larger C/EBPβ isoforms are associated with inhibition of proliferation and the expression of differentiation-related genes. Therefore, C/EBPβ may act as a guide in proliferation control, thereby enabling balanced differentiation. In addition, C/EBPβ may represent an important control system for the tightrope walk between physiologic/inflammatory proliferation and malignant proliferation, i.e. in AML. Another important function of the C/EBPβ system in monocytic cells is the orchestration and regulation of processes mainly related to innate immunity. During monocytic differentiation, and also upon activation by a variety of physiological and pathophysiological stimuli, C/EBPβ-dependent genes such as cytokines, chemokines, and phagocytosis-associated molecules are induced by LAP*/LAP which is itself limited by LIP. Dysregulation of C/EBPβ may result in the development and/or progression of severe disease states such as leukemia, sepsis, and microbial infection. Further studies are required to exactly characterize the role of C/EBPβ in monocytic proliferation/differentiation. In addition, experiments have to be performed to further elucidate the signaling modules as well as translational and proteolytic mechanisms leading to C/EBPβ expression. Finally, additional analyses are necessary to better conceive the involvement of the C/EBPβ system in inflammation and malignancy. Understanding the C/EBPβ system in monocytic cells may enable the development of diagnostic strategies to better explain on one hand the stages of physiologic and inflammatory proliferation and, on the other hand, malignant proliferation. In addition, therapeutic avenues may offer a feasible means to better control the excessive inflammation mediated by leukocytes as well as to specifically address distinct signaling modules in leukemia or antiviral therapy.

Conflict of interest

The authors declare that they have no conflict of interest.

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